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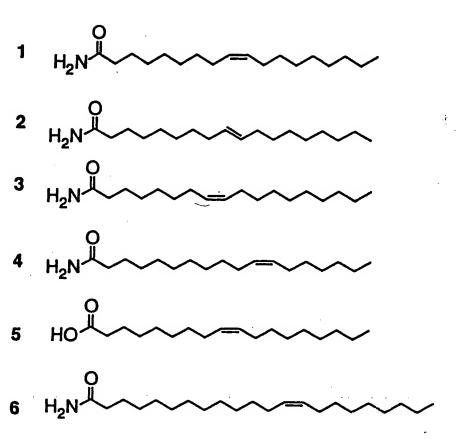
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SPGGSSGGEGALIGSGGSPLGLGTDIGGSIRFPSAFC
GICGLKPTGNRLSKSGLKGCVYGQTAVQLSLGPMARD
VESLALCLKALLCEHLFTLDPTVPPFPFREEVYRSSR
PLRVGYYETDNYTMPSPAMRRALIETKQRLEAAGHTL
IPFLPNNIPYALEVLSAGGLFSDGGRSFLQNFKGDFV
DPCLGDLILILRLPSWFKRLLSLLLKPLFPRLAAFLN
SMRPRSAEKLWKLQHEIEMYRQSVIAQWKAMNLDVLL
TPMLGPALDLNTPGR

### Rat Liver

- 1) Sucrose gradient of liver membrane
- 2) 100 mM Na<sub>2</sub>CO<sub>3</sub> wash
- 3) Solublization in Triton-based buffer

#### Liver Plasma Membrane

- 1) DEAE column (ion exchange)
- 2) Hg column
- 3) Heparin column (detergent xchng)
- 4) Affinity column with trifluoroketone inhibitor

Amidase activity 20-30 fold enriched, 10-15% yield

Assay used: 14C radiolabeled substrate and TLC analysis

Trifluoroketone Inhibitor:  $K_i = 1 \text{ nM}$ 

base deprotection of thioacetate, and immediate linkage

Link to disulfidederivatized solid support

$$F_3C$$
 (CH<sub>2</sub>)<sub>7</sub> (CH<sub>2</sub>)<sub>7</sub> S Beads

Advantage: thioacetate equivalent inhibitory potential to unmodified inhibitor, remove protein with reducing agent (20 mM DTT, 4° o/n)

FIG. 5

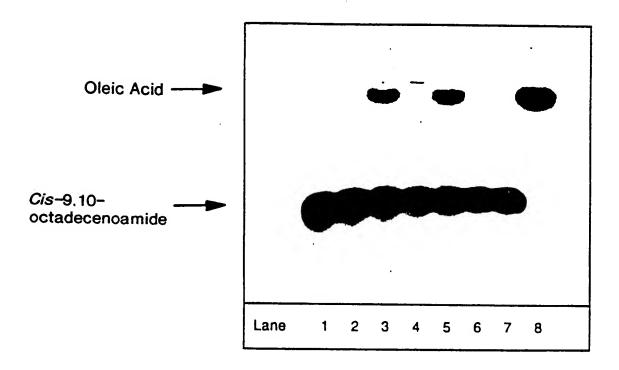


FIG. 6

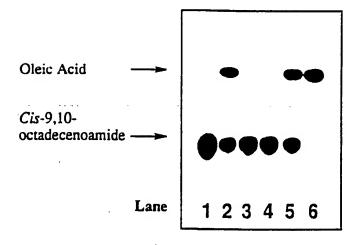


FIG. 7

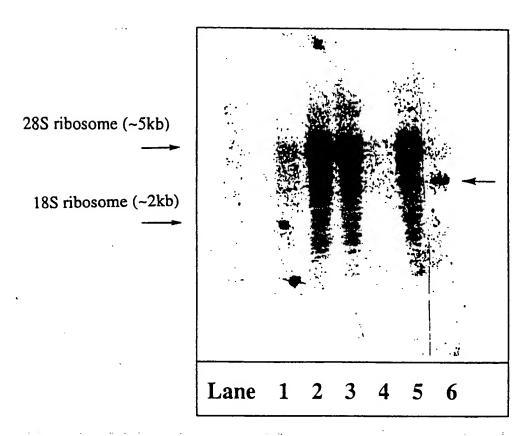


FIG. 8

1-MVLSEVWTTLSGVSGVCLACSLLSAAVVLRWTGRQKARGAATRARQKQRA
51-SLETMDKAVQRFRLQNPDLDSEALLTLPLLQLVQKLQSGELSPEAVFFTY
101-LGKAWEVNKGTNCVTSYLTDCETQLSQAPRQGLLYGVPVSLKECFSYKGH
151-DSTLGLSLNEGMPSESDCVVVQVLKLQGAVPFVHTNVPQSMLSFDCSNPL
201-FGQTMNPWKSSKSPGGSSGGEGALIGSGGSPLGLGTDIGGSIRFPSAFCG
251-ICGLKPTGNRLSKSGLKGCVYGQTAVQLSLGPMARDVESLALCLKALLCE
301-HLFTLDPTVPPLPFREEVYRSSRPLRVGYYETDNYTMPSPAMRRALIETK
351-QRLEAAGHTLIPFLPNNIPYALEVLSAGGLFSDGGRSFLQNFKGDFVDPC
401-LGDLILIRLPSWFKRLLSLLLKPLFPRLAAFLNSMRPRSAEKLWKLQHE
451-IEMYRQSVIAQWKAMNLDVLLTPMLGPALDLNTPGRATGAISYTVLYNCL
501-DFPAGVVPVTTVTAEDDAQMELYKGYFGDIWDIILKKAMKNSVGLPVAVQ
551-CVALPWQEELCLRFMREVEQLMTPQKQPS-579

FIG. 9

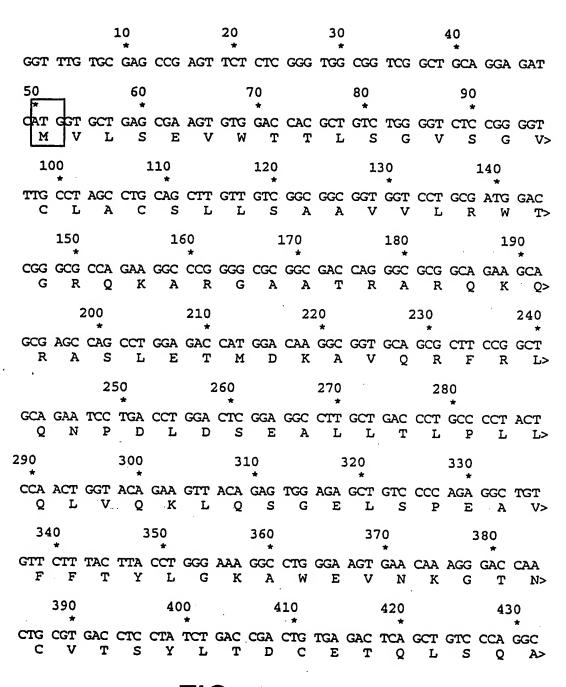


FIG. 10-1

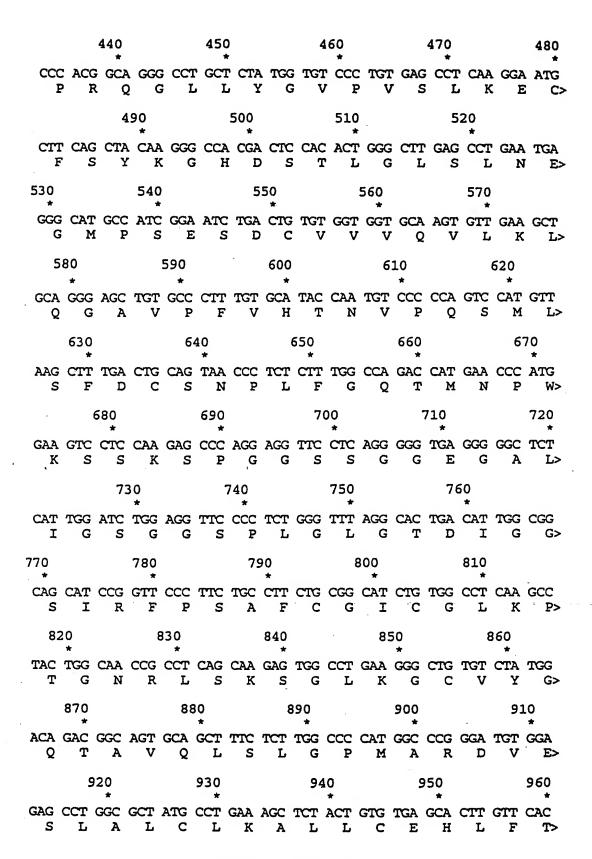


FIG. 10-2

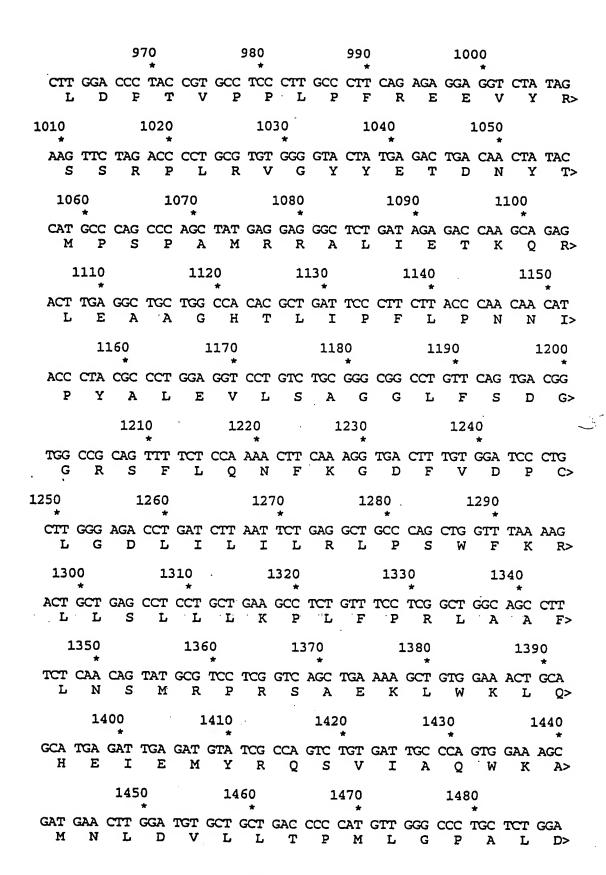


FIG. 10-3

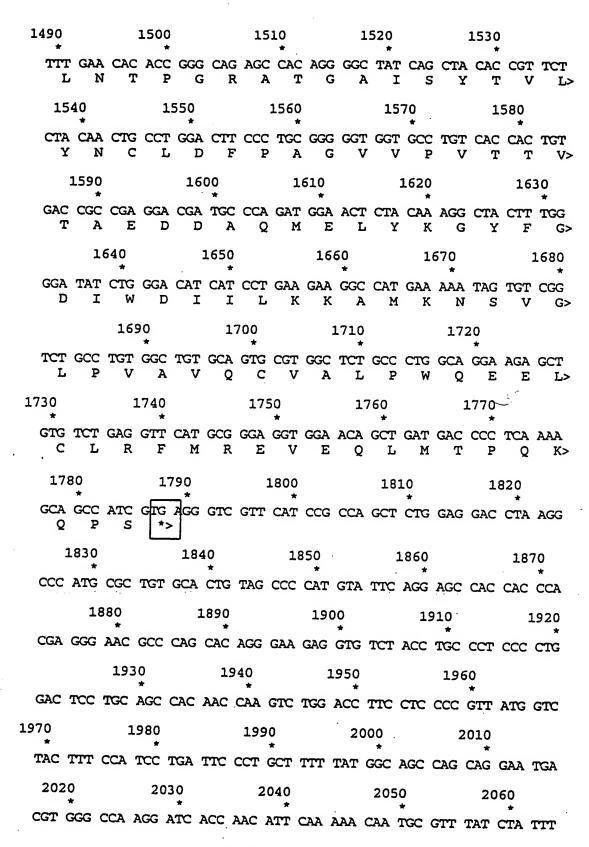
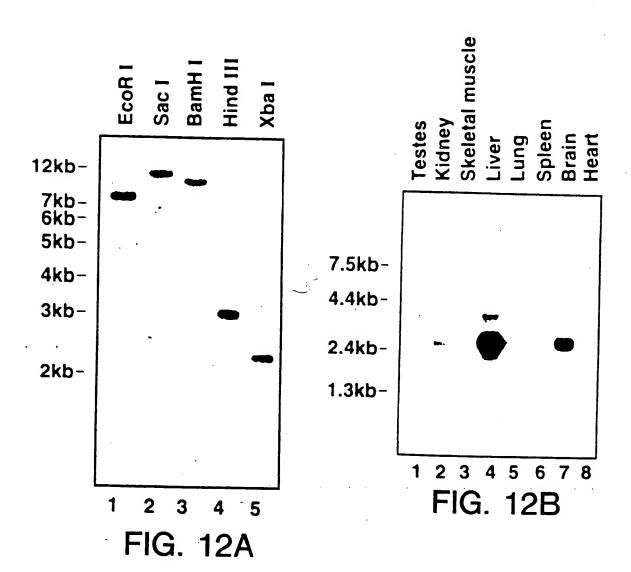


FIG. 10-4

2080 2090 2100 2110 TCT GGG TAT CTC CAT TAG GGC CCT GGG AAC CAG AGT GCT GGG AAG GCT GTC CAG ACC CTC CAG AGC TGG CTG TAA CCA CAT CAC TCT CCT GCT CCA AAG CCT CCC TAG TTC TGT CAC CCA CAA GAT AGA CAC AGG GAC ATG TCC TTG GCA CTT GAC TCC TGT CCT TCC TTT CTT ATT CAG ATT GAC CCC AGC CTT GAT GGA CCC TGC CCC TGC ACT TCC TTC CTC AGT CCA CCT CTC TGC CGA CAC GCC CTT TTT ATG GCT CCT CTA TTT GTT GTG GAG ACA AGG TTT CTC TCA GTA GCC CTG GCT GTC CAG GAC CTC ACT CTG TAG ATG AGG CTG 2410 2420 2430 GCT TTC AAC TCA CAA GGC TGC CTG CCT GGG TGC TGG GAT TAA AGG CGT ATG CCA CCA CAA AGA AAA AAA AAA

FIG. 10-5

Indoleacetamidase (Pseudomonas) Putative Amidase (S. cervevisiae) Putative Amidase (C. elegans) Propionamidase (Chick) Oleamide Hydrolase (Rat) Indoleacetamidase (Agrobacterium) Putative Amidase (C. elegans) Acetamidase (Aspergillus) 147-GGSSGGVAAAVASRLMLGGIGTDTGASVRLPA-178 207-GGSSGGEGSLIGAHGSLLGLGTDIGGSIRIPS-238 213-GGSSGGESALISADGSLLGIGGDVGGSIRIPC-244 222-GGSSGGEGALIAGGGSLLGIGSDVAGSIRLPS-253 215-GGSSGGEGALIGSGGSPLGLGTDIGGSIRFPS-246 144-GGSSGGVAAAVASGIVPLSVGTDTGGSIRIPA-175 202-GGSSGGEGAIVGIRGGVIGVGTDIGGSIDVPA-233 212-GGSSGGEGALIGAGGSLIGIGTDVGGSVRIPC-243



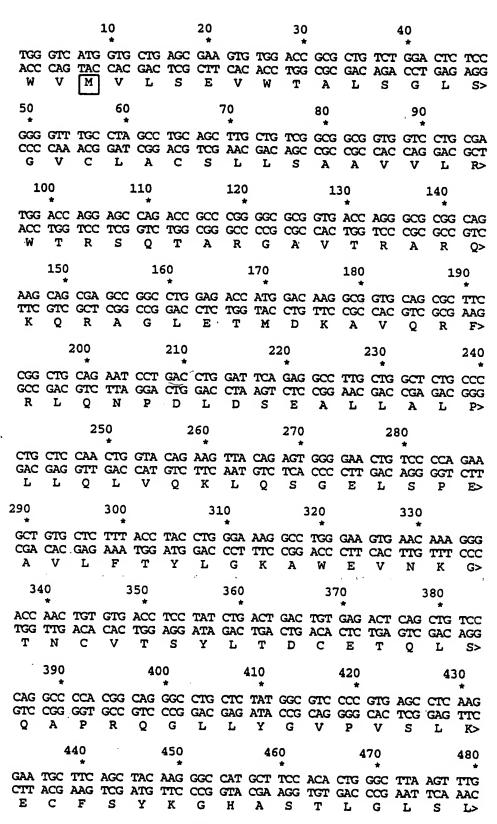


FIG. 13-1

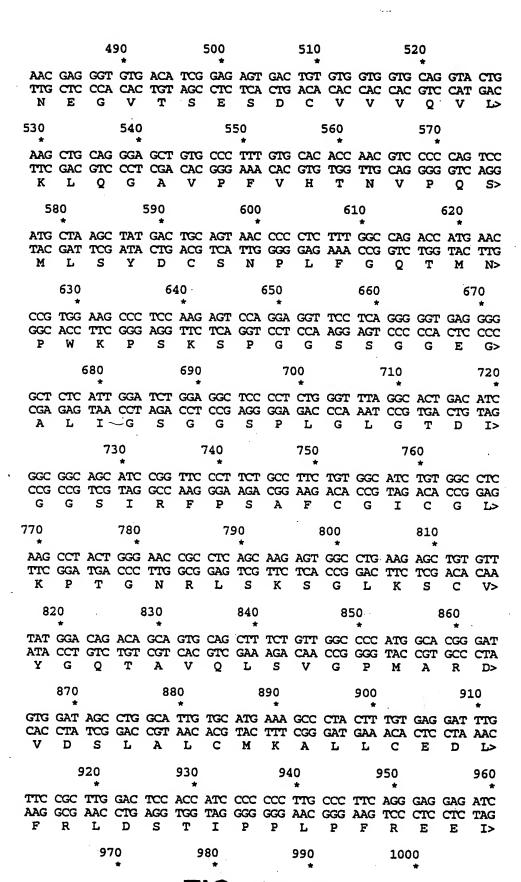


FIG. 13-2

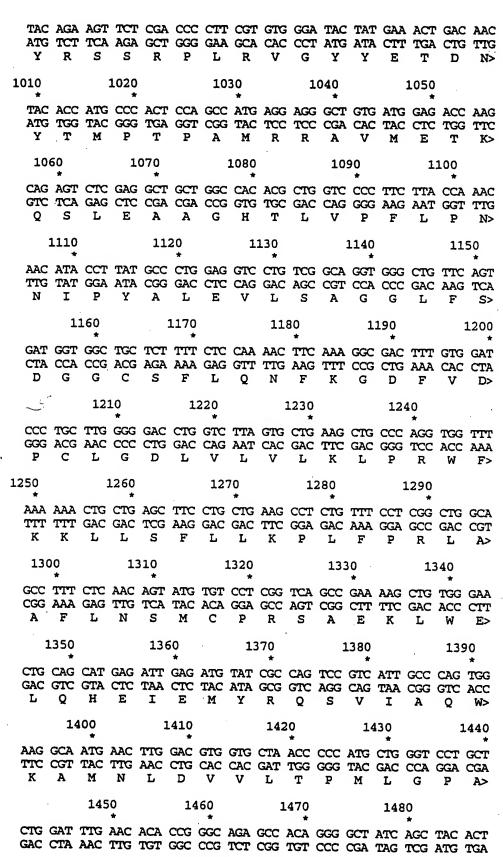


FIG. 13-3

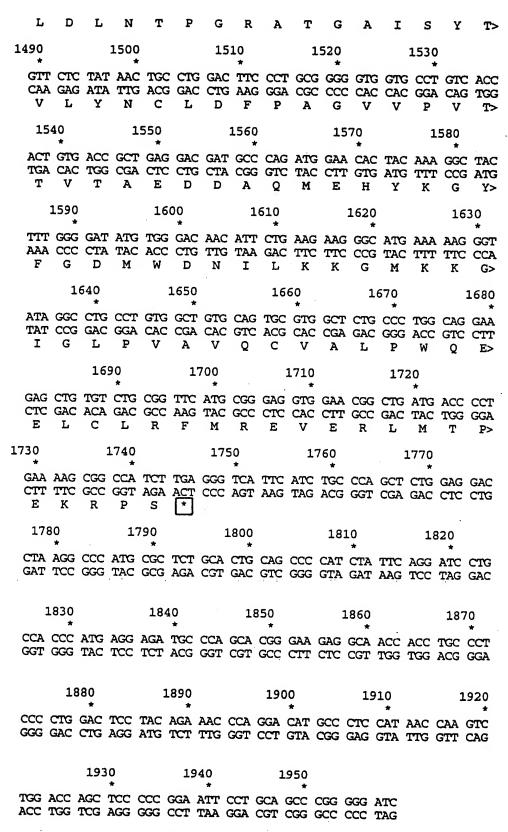


FIG. 13-4

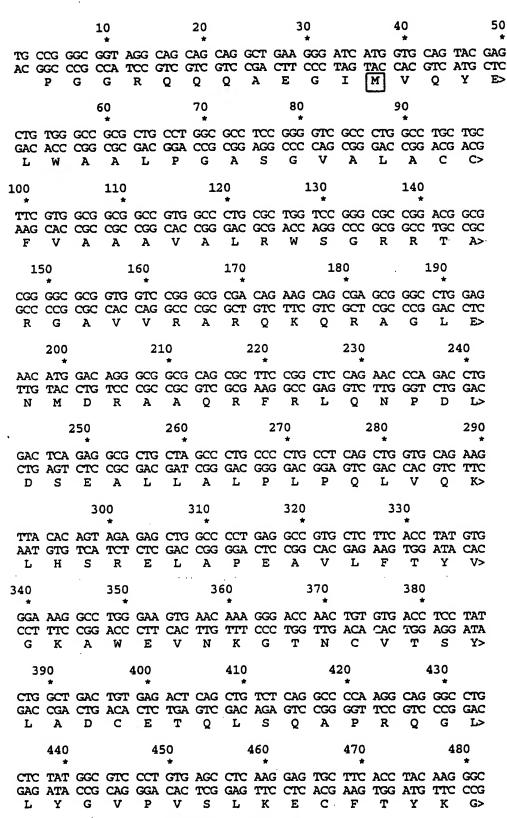


FIG. 14-1

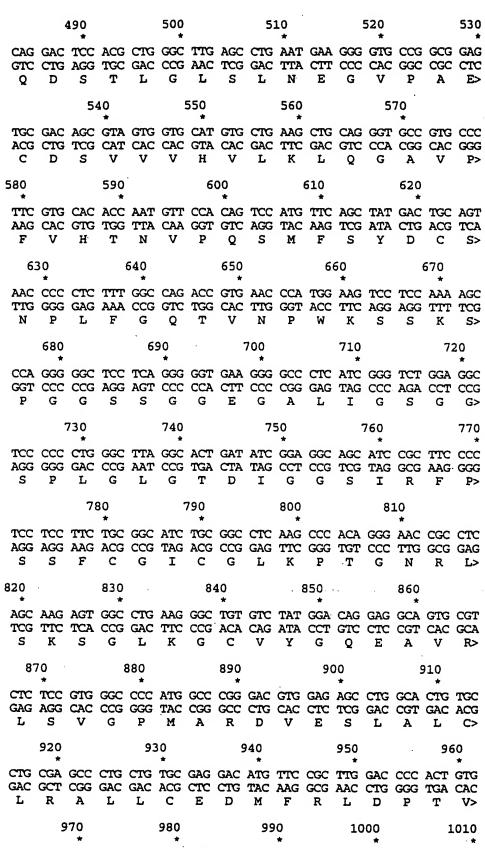


FIG. 14-2

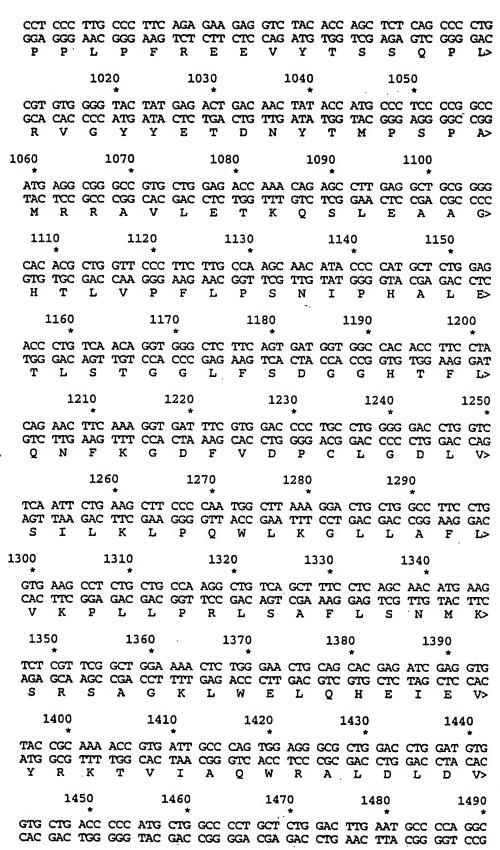
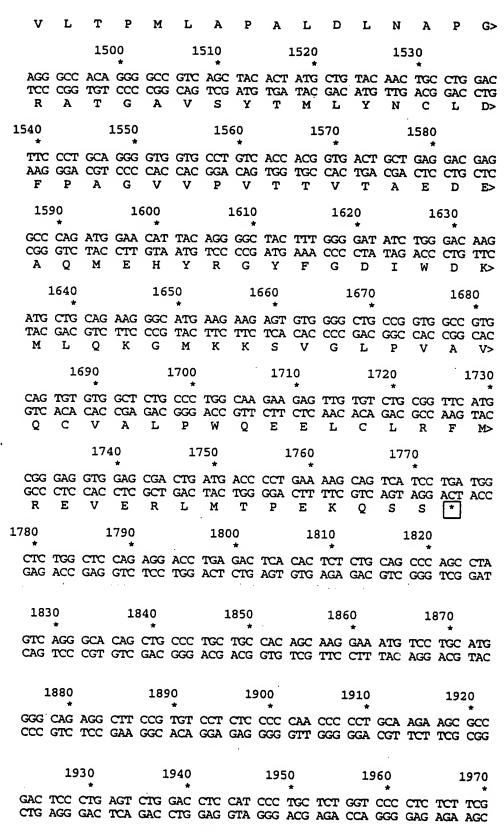


FIG. 14-3



Sec. 25.

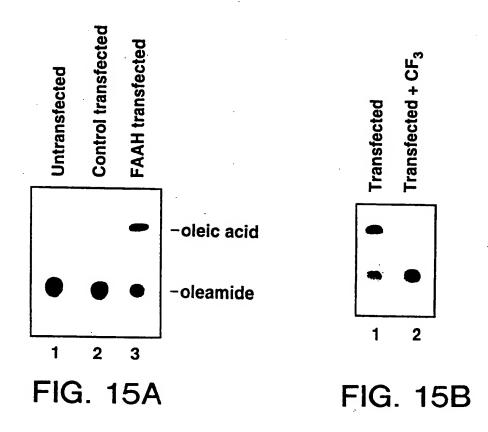
FIG. 14-4

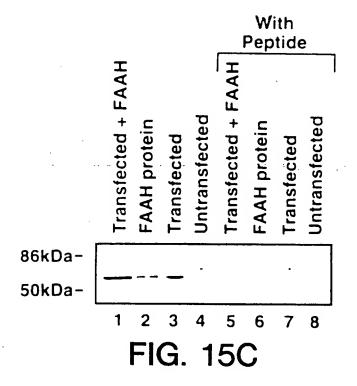
TCC TGA TCC CTC CAC CCC CAT GTG GCA GCC CAT GGG TAT GAC ATA GGC AGG ACT AGG GAG GTG GGG GTA CAC CGT CGG GTA CCC ATA CTG TAT CCG

Marita de la composição d

CAA GGC CCA ACT AAC AGC CCC GGA ATT GTT CCG GGT TGA TTG TCG GGG CCT TAA

FIG. 14-5

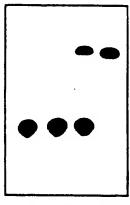




- oleic acid
- − oleamide
- 1 2

FIG. 16

Anandamide std. Untransfected FAAH transfected Arach. acid std.



1 2 3 4